l agggagagge agtgaceatg aaggetgtge tgettgeeet gttgatggea 51 ggcttggccc tgcagccagg cactgccctg ctgtgctact cctgcaaagc 101 ccaggigage aacgaggact gcctgcaggt ggagaactgc acccagctgg 151 gggagcagtg ctggaccgcg cgcatccgcg cagttggcct cctgaccgtc 201 arcagcaaag gergeagett gaactgegtg gatgaeteae aggaetaeta , 251 cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgcca 301 geggggeeca tgeeetgeag eeggetgeeg ceateettge getgeteect 351 geacteggee tgetgetetg gggaceegge eagetatagg etetgggggg 401 ccccgctgca gcccacactg ggtgtggtgc cccaggcctt tgtgccactc 451 ctcacagaac ctggcccagt gggagcctgt cctggttcct gaggcacatc 501 ctaacgcaag tttgaccatg tatgtttgca ccccttttcc ccnaaccetg 551 acctteceat gggeetttte eaggatteen acenggeaga teagttttag 601 tganacanat ccgcntgcag atggcccctc caaccnttin tgttgntgtt 651 tecatggeee ageattitee accettaace etgtgtteag geactinite 701 ccccaggaag concectge ccaececan tatgaanga gccaggmg 751 greegragig tecceogeae ceageagaga acaagacaate aggaagageee 801 agiaaagget gagatgaagt ggaetgagta gaactggagg acaagagtig., 351 acgigagito oigggagitt ocagagatgg ggcoiggagg coiggaggaa 901 ggggccaggc creacattig iggggnicee gaatggcage etgagcaeag 951 egizggeet taataaacae etginggata agecaaaaaa aaaaaaaa

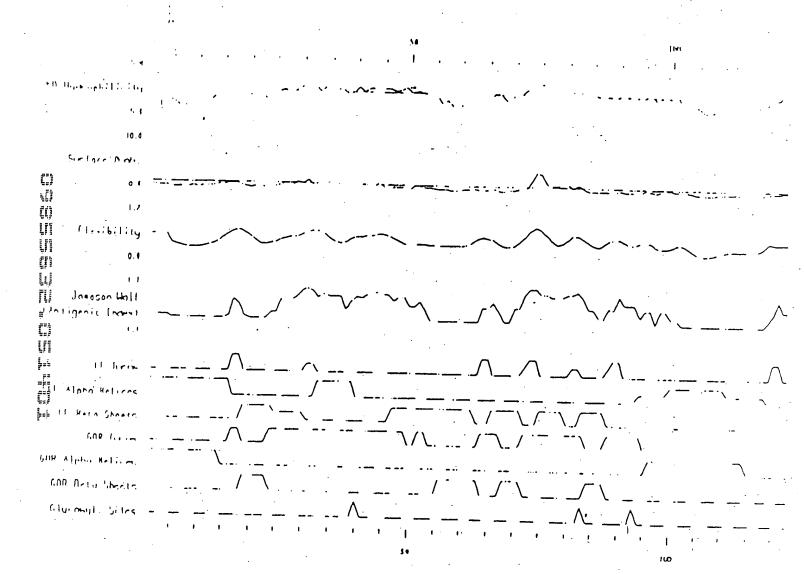
MARTULALLMAGLALQPGTALLCYSCKAQVSNEDCLQVENCTQLGEQCWTARIRAVGLLTV I SKGCSLNCVDDS
ODYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPAL

FIGURE 1B

						•																
	A 1 -	TGA	AGA	CAG	TTT	TTT	TTA	TCC	TGC	TGG	CC	CCI	'ACI	TAG	CC	CTG	CA'	rcc	AGO	GTG	CTGC	T
																					GACG	
	M			V	F	•	_						L				ł	P	G	A	Α	-
61	CTGCAGTGCTATTCATGCACAGCACAGATGAACAACAGAGACTGTCTGAATGTACAGAAC															:						
																					CTTC	
	Ľ	Q	C	Y	s	С	T	A				N		D		L		N	v	Q	N _i	
121	TGCAGCCTGGACCAGCACAGTTGCTTTACATCGCGCATCCGGGCCATTGGACTCGTGACA																					
											• • • •										CTGT	
,	Ç	s	. L	D	Q	Н	s	С	F	T	s	R	I	R	A	I		G	L	v	т	
181	`G1	TAT	CAC	TAP	GGC	СТС	CAG	CTC	ACA	GTC	TG	\GG2	ATG/	CTO	GG.	AGA	AC	TAC	CTA'	TTT	GGGC	
	CA	ATA ·	GTC	LATT	CCC	GAC	GTC	GAG	TGT	CAC	ACI	CCI	CACT	GAC	CC.	rct	TG.	ATC	AT	AAA	CCCG	240
241	V	I	s	K	G	C	s	s	Q	С	E	D	D	s	E	N		Y	Y	L	G	-
	AA	GAA	GAA	CAT	CAC	GTG	CTG	CTA	ĊTC	TGA	CCI	GTO	CAA	TGT	CA	ACG	GG	GCC	CAC	CAC	сста	
																300						
	K	K.	N	I	T	C	С	Y	s	D	L	С	N	v	N	G	1	Ą	н	T	L	
301	ĄĄ	AAGCCACCCACCCTGGGGCTGCTGACCGTGCTCTGCAGCCTGTTGCTGTGGGGCTCC																				
	·															360						
	K	P	P	T	-	L	G	L	Ĺ	T	v	L	c.			. L	L			G	s	
361	AG	CCG	rc T	GTA(GC.	TCT	GGG	AGAC	GCC.	rac(CAT	AGC	CCG,	ATT	GTG	AAC	GG	АТ	GAG	СТС	CAC	. `
201		AGCCGTCTGTAGGCTCTGGGAGAGCCTACCATAGCCCGATTGTGAAGGGATGAGCTGCAC 1CGGCAGACATCCGAGACCCTCTCGGATGGTATCGGGCTAACACTTCCCTACTCGACGTG														420						
	S		L																			
421	TCC	CACC	CCA	ACC	CCA	ACAC	CAGO	, , .								-				•		i
	AGC	TGC	GG1	GGC	GG1	CTC	TCC	44	1													•

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1 MIRITUP VILLAGUE ING V S RASS
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1 MRAVILLA IL MAG LA LO DOG TA
21 IL C S SIC L N O K S IN L Y C I K P T I
21 IL C Y S C X A O V S N S D C I Q V E N P
21 L Q C Y S C T A Q M N N R D C L N V Q N P
41 C S O O D N Y C V T V S A S X G I G N L
41 C T O L G E Q C M T A R I R A V G L L T
41 C S L D Q M S C F T S R I R A I G L V T
61 V T F G H S L S X T C I S P A C P I P E G
61 V - - - - I S K G C S L N C V D D S Q
61 V - - - - I S K G C S L N C V D D S Q
61 V - - - - I S K G C S L N C V D D S Q
61 V - - - - I S K G C S L N C V D D S Q
61 V - - - - I S K G C S L N C V D D S Q
61 V - - - - I S K G C S C S C S C S D C E D D S S
81 V N V G V A S M G I S C C Q S F LI C N P
76 D Y Y V G K K - N L T C C D T D L C N P
76 N Y Y L G K K - N I T T C C Y S D T C N P
101 S A A D G G L R A S V T I T G A G I I L
95 S G A H A L D P A A A I L A L L P A F G
95 N G A H T L X P P T T L G L E T Y L C S
121 S L I L A G S S R L - -
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> = 9/4cosylation site

J GPI signa

prostate (Buth)
prostate (Buth)
prostate (Buth)
Bladder (Huner)
Bladder (dek)
Bladder (Rd)
Kidney (NLO)
Kidney (NLO)
Kidney (NLO)

Kidney (NLO)

Fostis
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LAPCI

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Tij

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translated grayion of PSCA legent: [::] untranslated exon 3 exon 4 Ex2 Ex3 exon 2 AIG ATG Ex1 exoń 1 8B F1G. 8A FIG. 8C 7,66 988 تَحِيرُ FIGURE 8

Mary Co

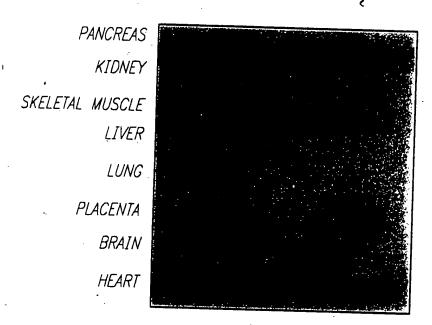
Erz Er3

EXI

PSCA / PSA Expression in Benign Prostate vs. Prostate Cancer Xenograft

Berlief April Apri

PSCA PSA



PERIPHERAL LEUKOCYTES

COLON

SMALL INTESTINE

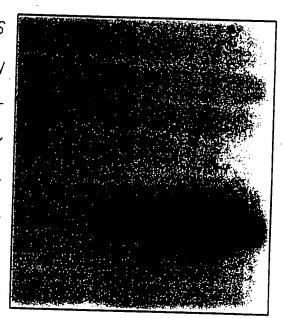
OVARY

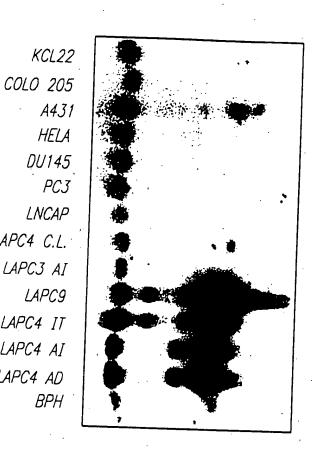
TESTIS

PROSTATE

THYMUS

SPLEEN





COLO 205

A431

HELA

DU145

PC3

LNCAP

LAPC4 C.L.

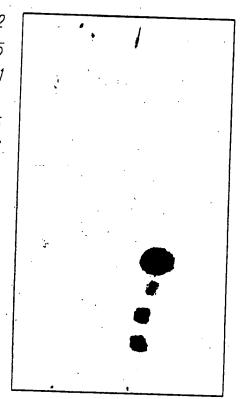
LAPC3 AI

LAPC9

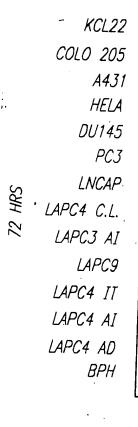
LAPC4 AI

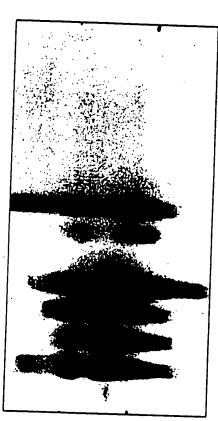
LAPC4 AD

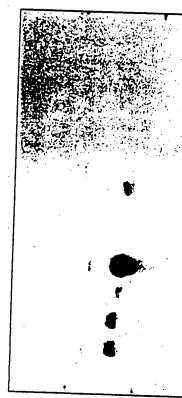
BPH



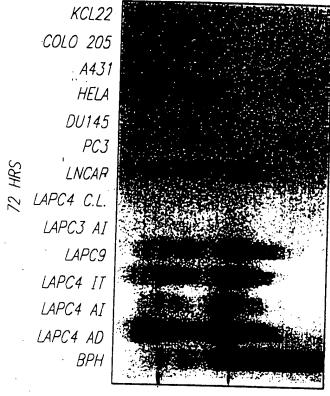
PSCA







KCL22 COLO 205 A431 HELA DU145 PC3 LNCAP LAPC4 C.L. LAPC3 AI LAPC9 LAPC4 IT LAPC4 AI LAPC4 AD . BPH



HELA DU145 PC3

LNCAP

LAPC4 C.L.

LAPCJ AI

LAPC4 IT

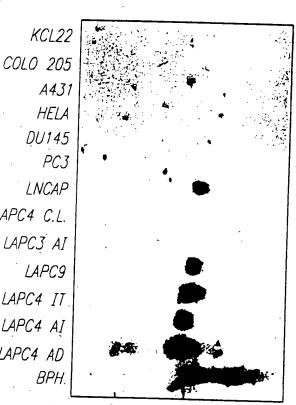
LAPC4 AI

LAPC4 AD

ВРН.

PSA

LAPC9



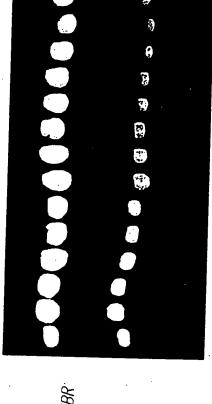


FIG. 11A





FIG. 11B

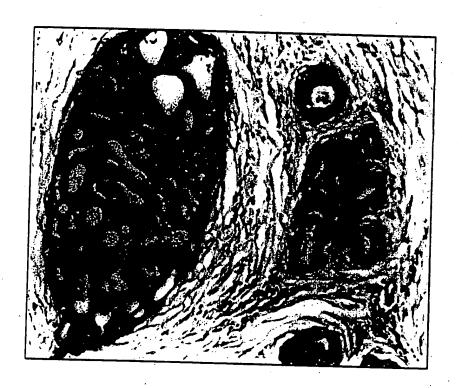
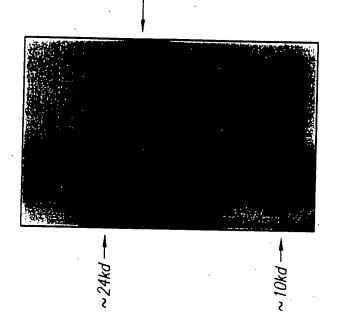


FIG. 11C



CELL ASSOCIATED

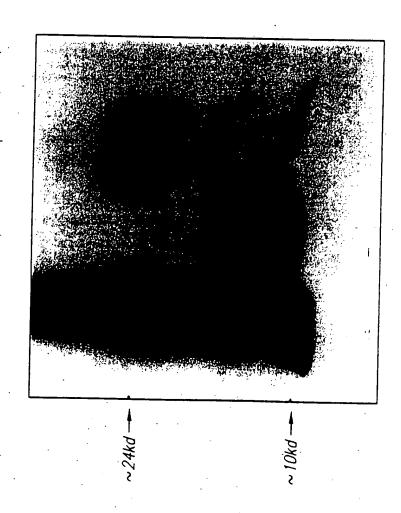


O GLYCOSIDASE

N GLYCOSIDASE F

CONTROL

FIG. 12,



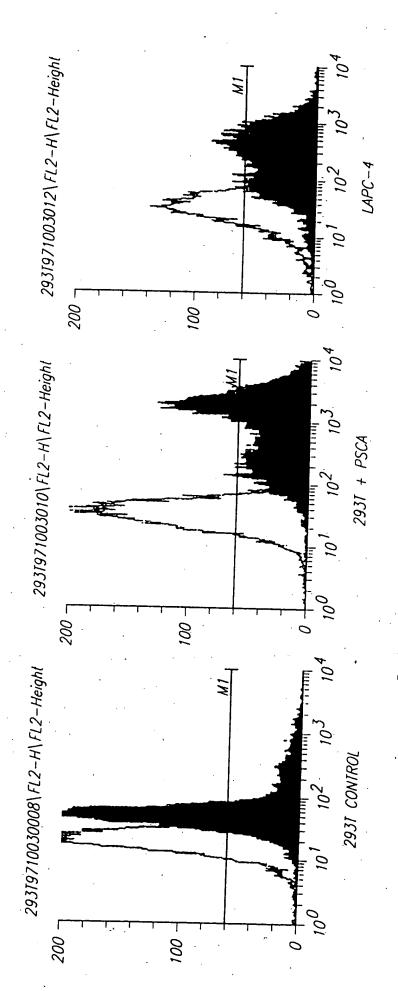


FIGURE 12C

PSCA Maps to Chromosome 8q24.2



And July that the mail mad that

Fluorescent in Situ Hybridization Analysis of PSCA

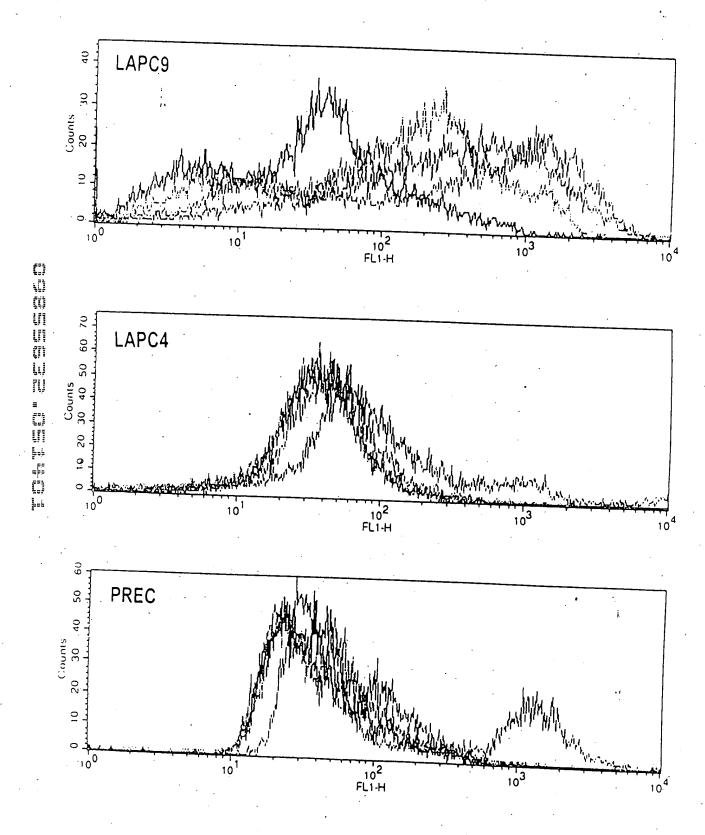


FIGURE 14

C. (85-123) 0.000 0.021 0.005 0.370 0.014 0.003
M (46-109) 0.628 0.032 0.016 0.000 0.000
N (2-50) 0.007 0.863 1.965 0.024 1.315 0.733 1.731
EL (18-98) 2.039 1.318 2.893 0.328 2.039 1.366 2.805
g ~~~~~~~
Isotype IgG1 IgG2a IgG3 IgG2a IgG2a IgG2a IgG2a
mAb 1G8 2H9 3C5 3E6 4A10 2A2 3G3

2A2 168

3C5

2H9

U ∑ Z

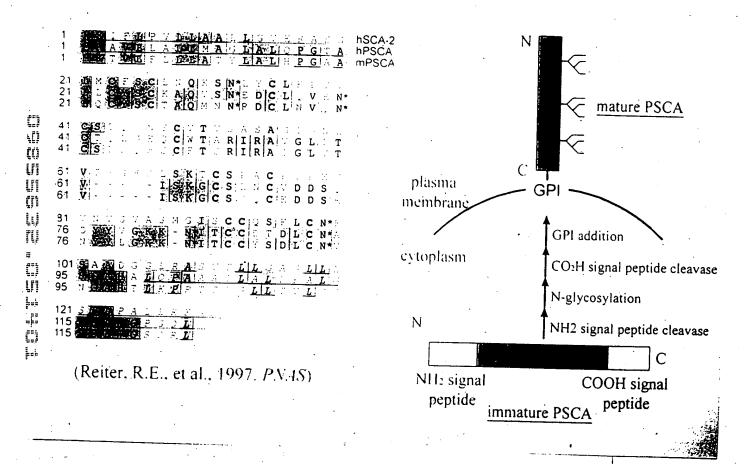
υ Σ Z 3G3

S Z Z

4A10

FIGURE 15

Prostate Stem Cell Antigen (PSCA) is a GPI-anchored Protein



R. Jenkins

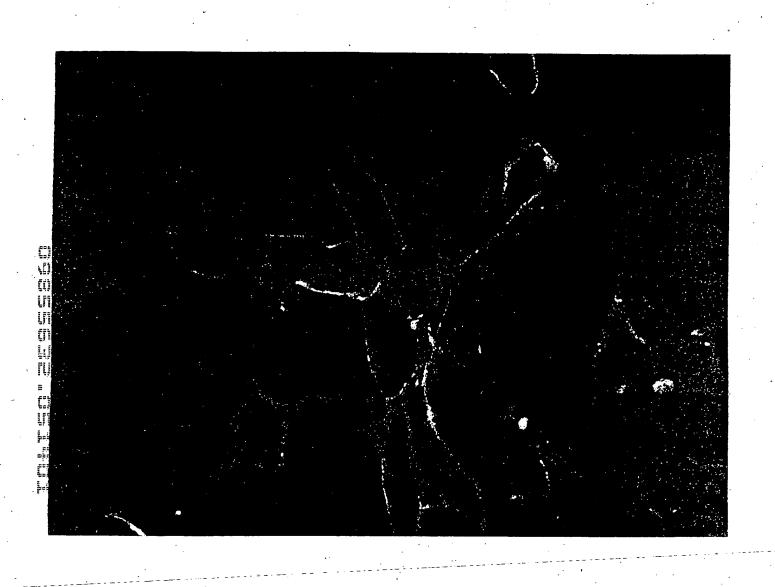


FIGURE 18





FIGURE 20

PSCA Immunostaining of Primary Tumors

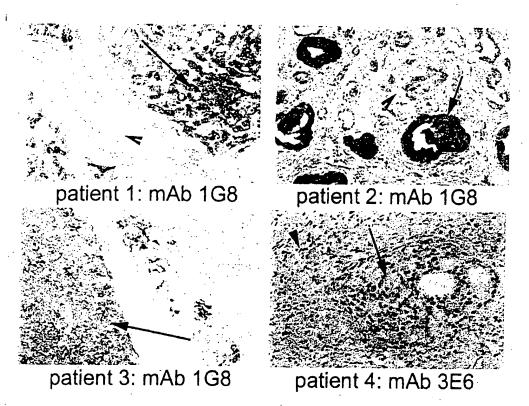




FIGURE 22



FIGURE 23



FIGURE 24

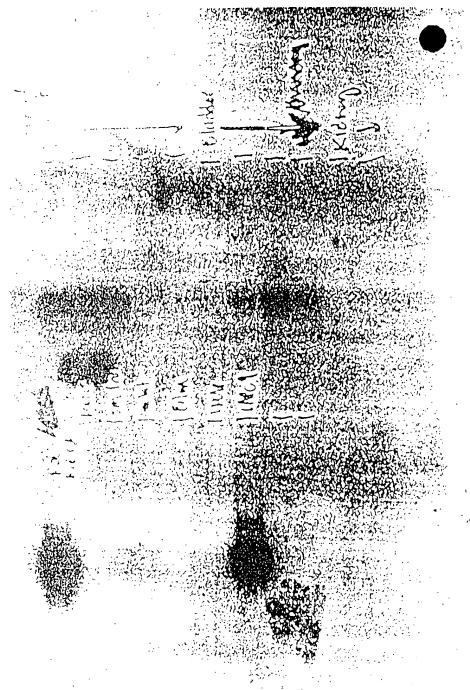


FIGURE 25

FIGURE 26

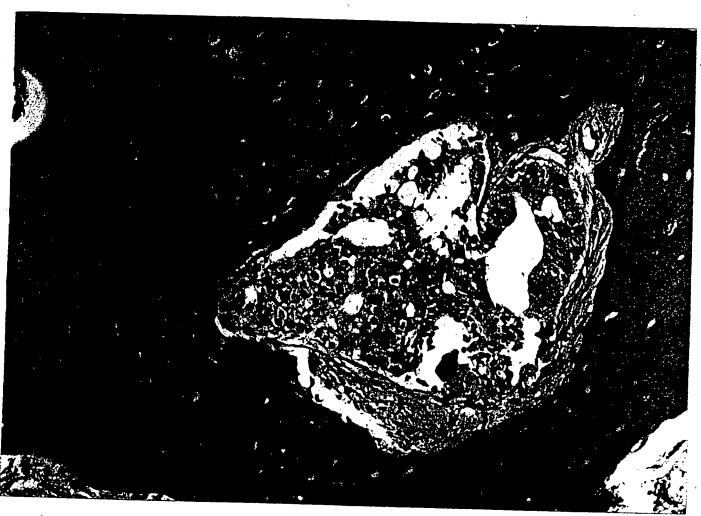


FIGURE 27

Patient 5: H and E and mAb 1G8

Patient 4: H and E and mAb 3E6

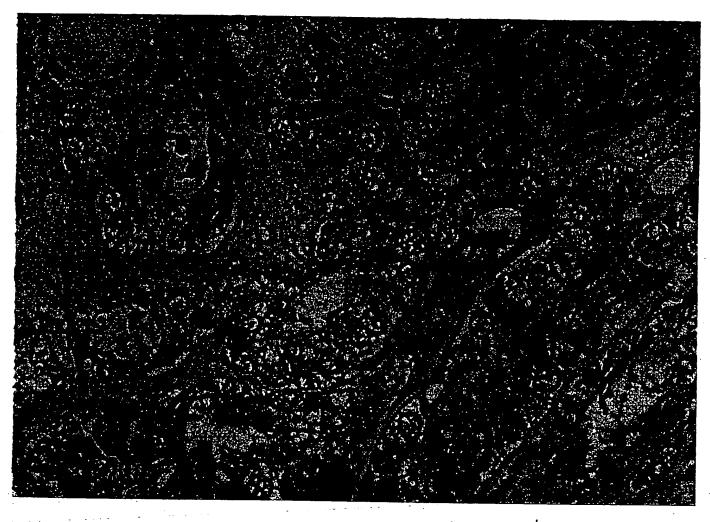


FIGURE 29

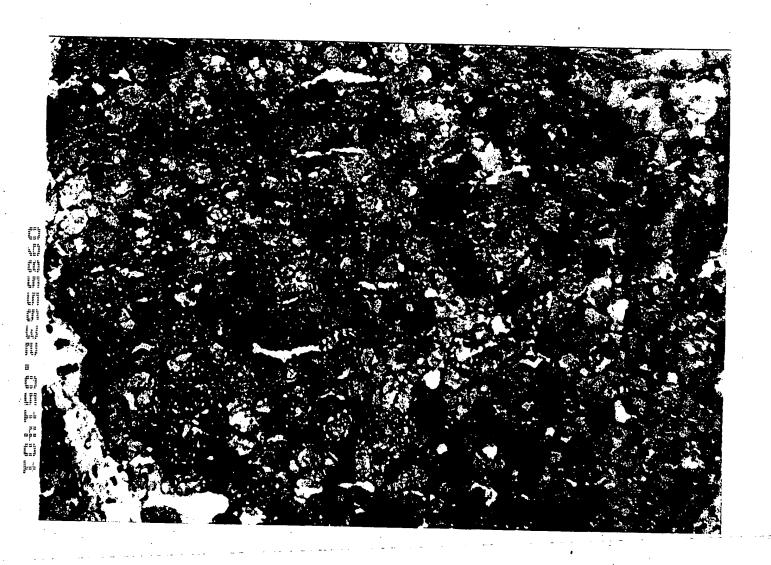


FIGURE 30

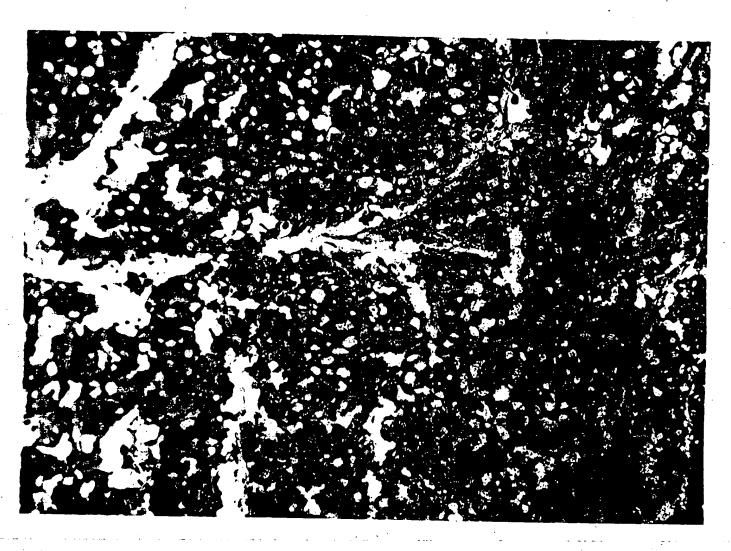


FIGURE 31

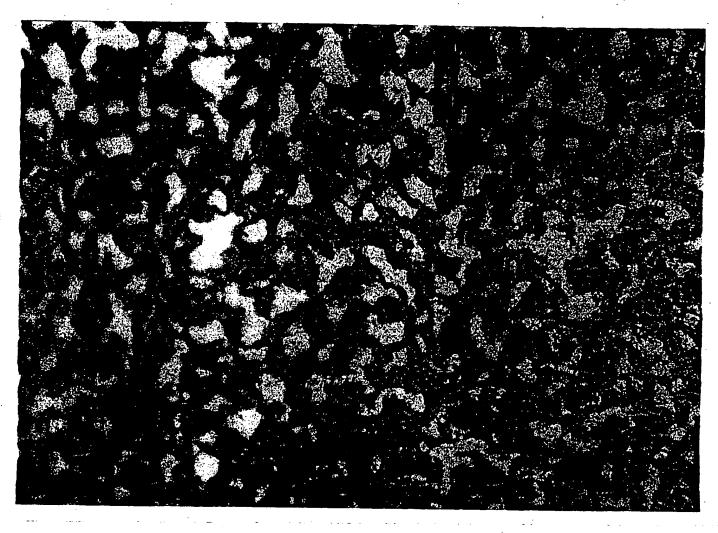


FIGURE 32

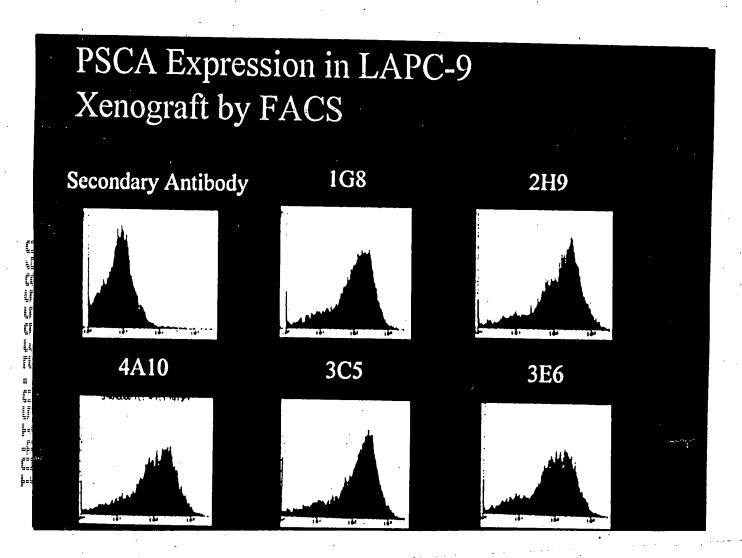


FIGURE 33

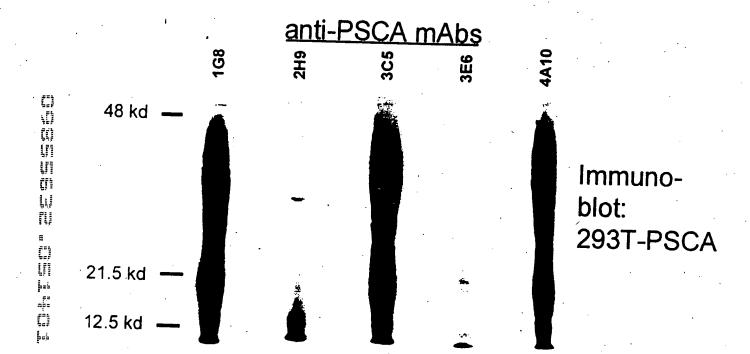


FIGURE 34

Immunofluorescent Staining of LNCaP-PSCA Cells

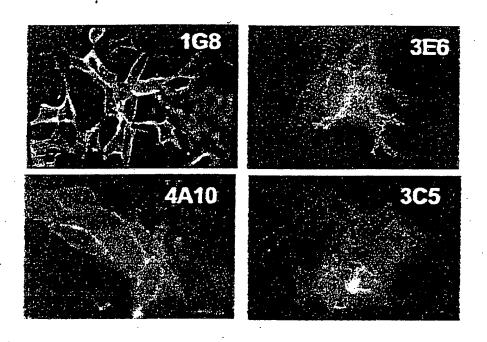
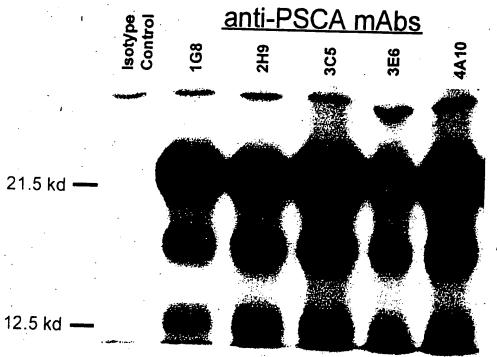




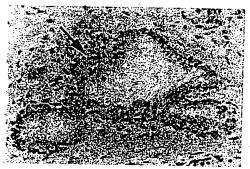
FIGURE 36



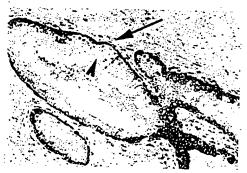
Immunoprecipitation: 293T-PSCA

Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6

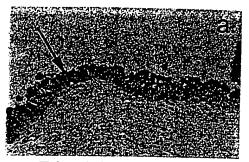


Normal: PSCA mAb 1G8 Atrophy: PSCA mAb 2H9

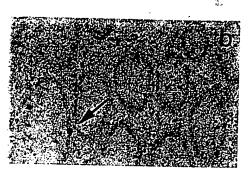




ŀΑ.



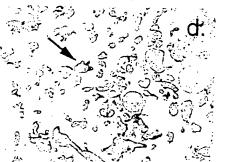
Bladder: 1G8



Colon: 1G8

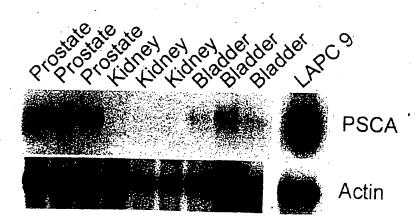


Kidney: 3E6

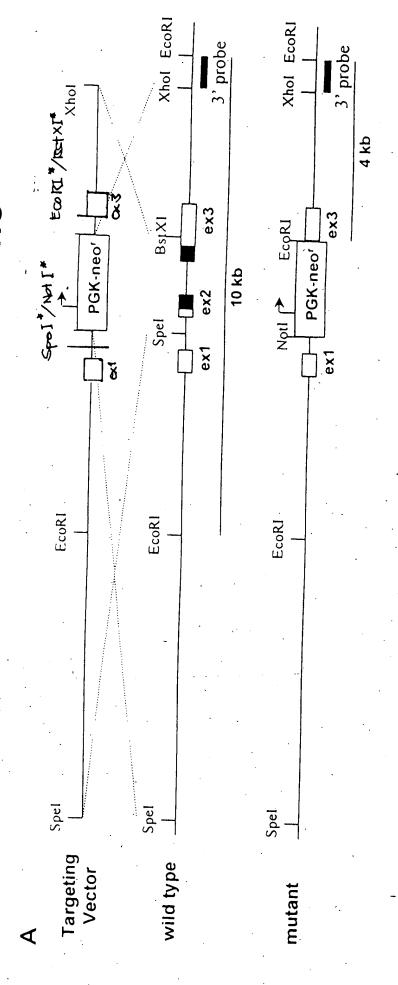


Placenta: 3E6

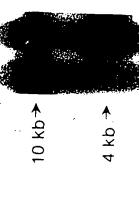
B.



Targeting of Mouse PSCA Gene



B. Genomic Southern Analysis of ES Cells -/+ +/+ * ex1, 2, and 3 are the exons of PSCA gene. * Black boxes of ex2 and ex3 encode PSCA mature protein sequences.

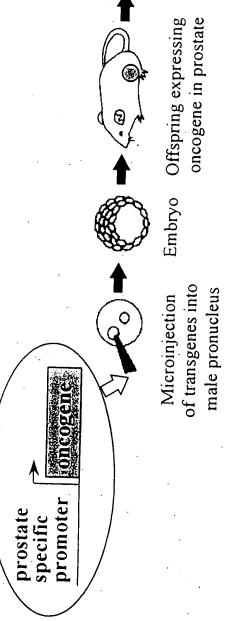


* ES genomic DNA's were digested with EcoRI, followed by Southern hybridization

using 3' probe

FIGURE 40

Transgenic Mouse Models of Prostate Cancer

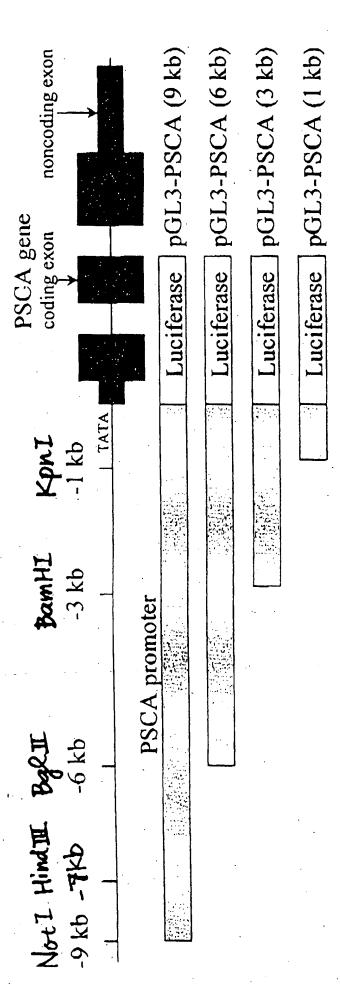


Mouse bearing	prostate cancer
Mor	pros

3

Characteristics	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcicinoma 28 wks No metastases	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcicinoma 12 wks Metastases in lymph node,	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcicinoma 16 wks Metastases in lymph node, lung, liver and bone
Target tissues	prostate (secretory cells) urethral, mammary and sweat gland	prostate (secretory cells)	 prostate (neuroendocrine cells) small intestine
Transgene	C3(1) (-3 kb)/ SV40 large+small _l T Maroulakou et al. 1994 PNAS	Probasin (-426 bp)/ SV40 large+small, T Greenberg et al. 1995 PNAS	Cryptdin2 (-6.5 kb)/ SV40 large+small, T Garabedian et al. 1998 PNAS

Reporter Gene Constructs for Transfection Assay

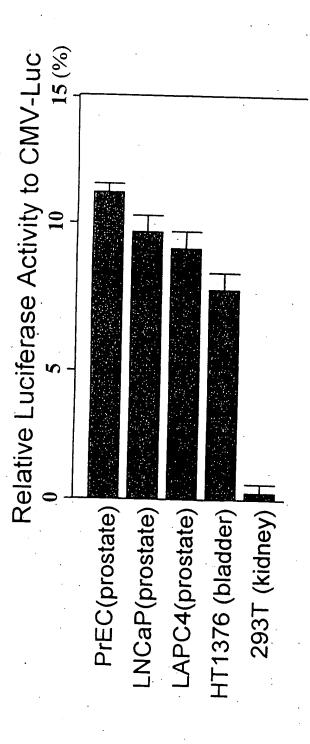


Luciferase pGL3-CMV

| pGL3-basic

Luciferase

CMV promoter



Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

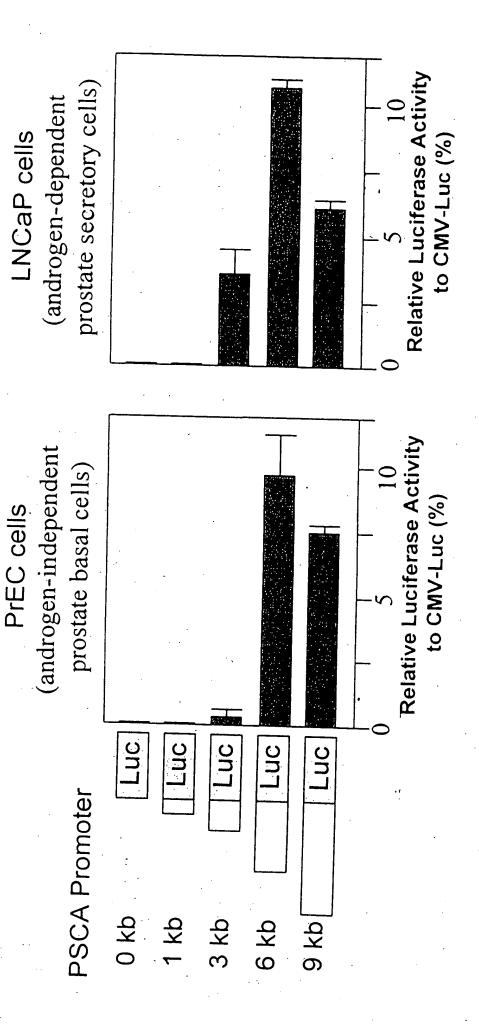
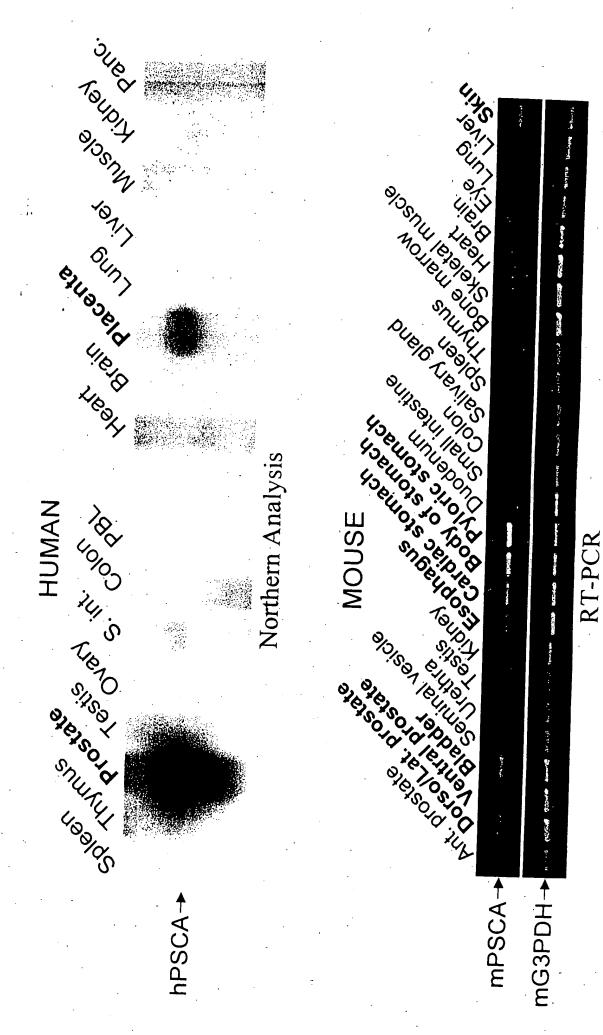


FIGURE 44

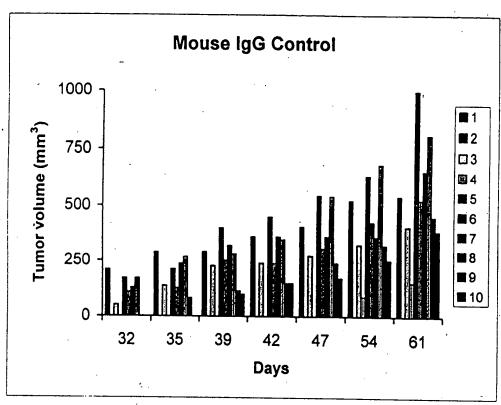
Update of Transgenic Mouse Projects

(DNA positive) Number of Founders Genomic Structure of PSCA exon 1 exon 2 exon 3 PSCA promoter (6kb) (CEP) 3, PCH intron GRE DA PSCA promoter (6kb) **(GBB) DA** ATG PSCA promoter (6kb) PSCA promoter (9kb) PSCA promoter (9kb) PSCA promoter (9kb) PSCA promoter PSCA(9 kb)-GFP-3'hGH PSCA(6 kb)-GFP-3'hGH PSCA(9 kb)-SV40TAG PSCA(6 kb)-SV40TAG PSCA(6 kb)-GFP PSCA(9 kb)-GFP

Whole-mount green fluorescence image Non-transgenic Transgenic (A25-106-2)(A25-106-2)Prostate (A25-104)Bladder Skin Negative tissues Seminal Vesicle Skeletal muscle Small intestine Stomach Urethra Kidney **Testis** Colon Ovary Uterus Liver Lung Brain Heart



MGUIRE 47



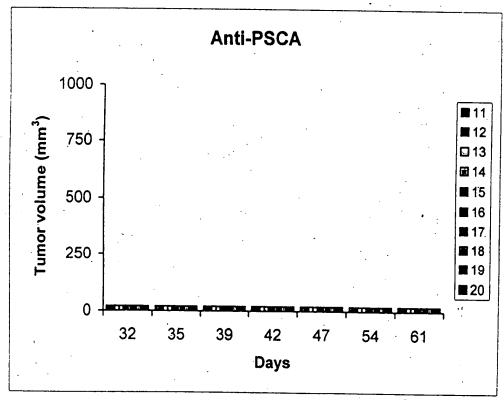
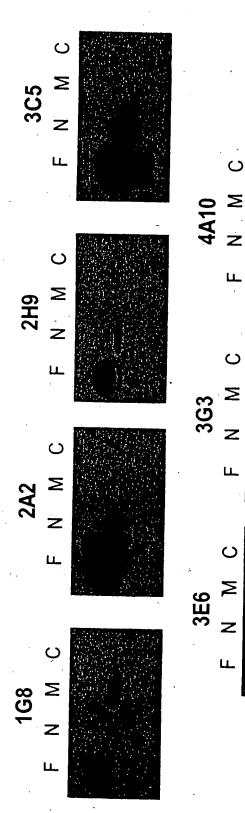
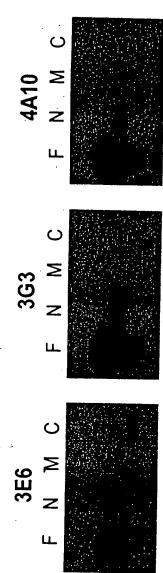


FIG. 49

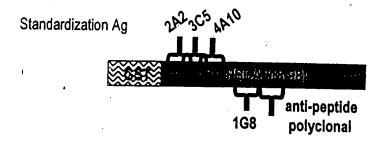
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ubc
recogi
obe
Epit

j.						
0.003	0.010	0.001	0.002	2.118	0000	0.001
1.273	0.023	0.002	900.0	1.133	0.004	0.000
0.004	0.631	1.026	1.709	0.036	1.731	0.493
1.485	0.973	1.069	1.916	1.609	2.805	1.053
lgG1 k	lgG2a k	lgG1 k	lgG2a k	lgG3 k	lgG2a k	lgG2a k
168	2A2	2H9	3C5	3E6	363	4A10
	lgG1 k 1.485 0.004 1. 273	lgG2a k	lgG1 k 1.485 0.004 1.273 lgG2a k 0.973 0.631 0.023 lgG1 k 1.069 1.026 0.002	lgG2a k	lgG1 k 1.485 0.004 1.273 lgG2a k 0.973 0.631 0.023 lgG1 k 1.069 1.026 0.002 lgG2a k 1.916 1.709 0.006 lgG3 k 1.609 0.036 1.133	1G8 IgG1 k 1.485 0.004 1.273 0.003 2A2 IgG2a k 0.973 0.631 0.023 0.010 2H9 IgG1 k 1.069 1.026 0.002 0.001 3C5 IgG2a k 1.916 1.709 0.006 0.002 3E6 IgG3 k 1.609 0.036 1.133 2.118 3G3 IgG2a k 2.805 1.731 0.004 0.000

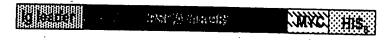




A



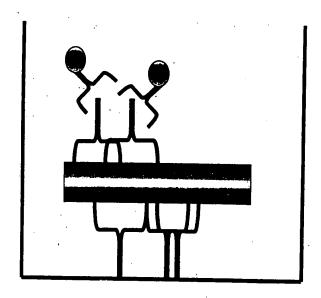
Engineered mammalian secreted form



B

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dret, men. ... n. o. den. N. H. Bess. O W.H. h H Heaft milh H H_{eaf}p

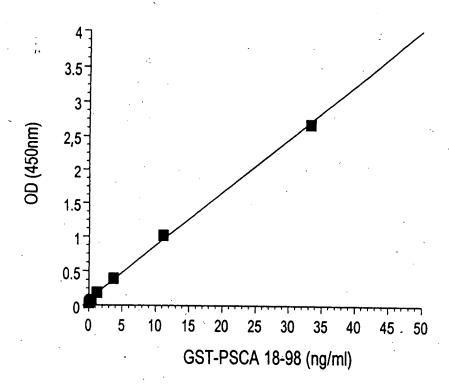


Anti-IgG2a HRP

Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

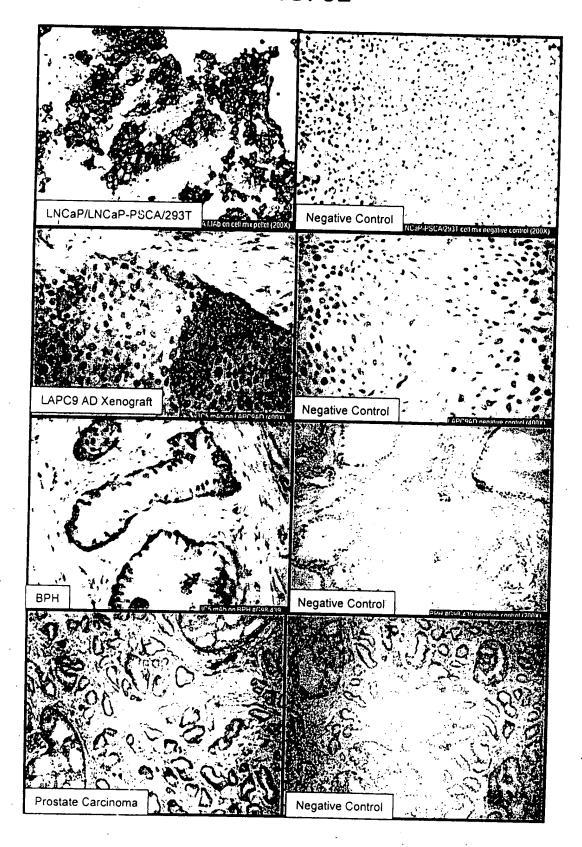
Affinity purified anti-peptide polyclonal + mAb 1G8 (IgG1)

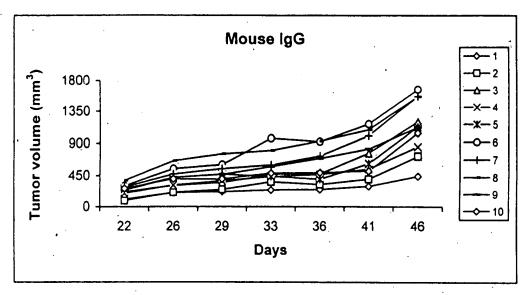


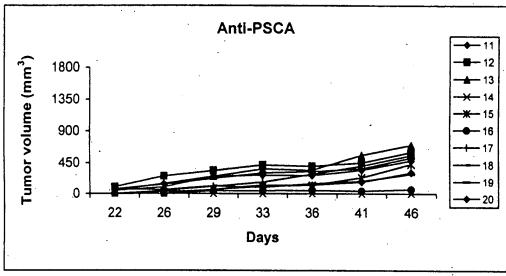
B

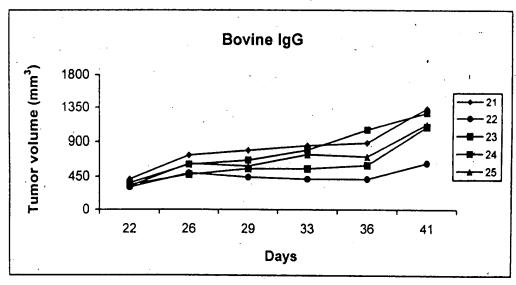
<u>Sample</u>	OD+range (n=2)	ng/ml
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND'
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

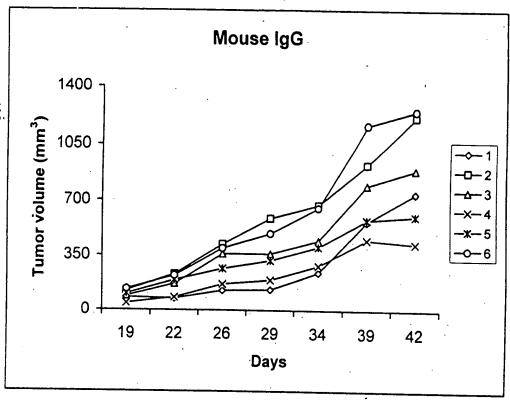
FIG. 52

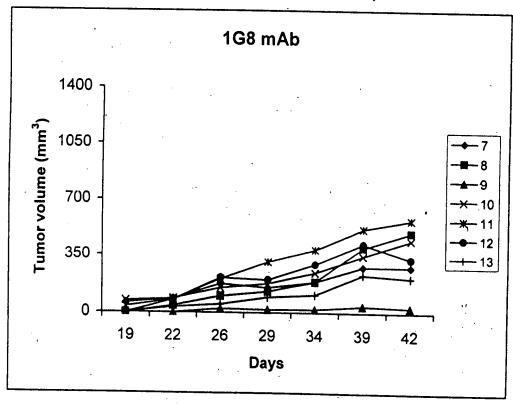


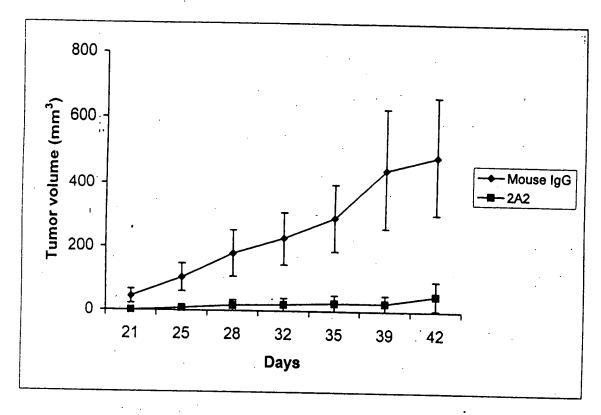


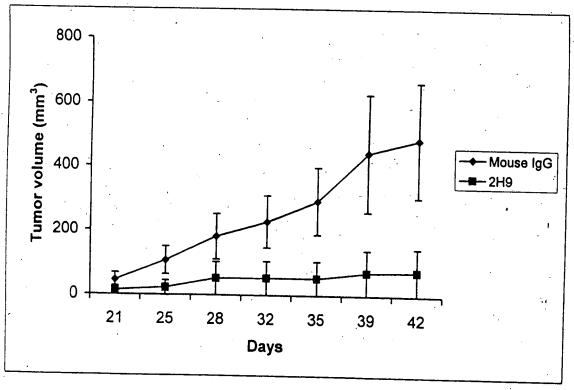




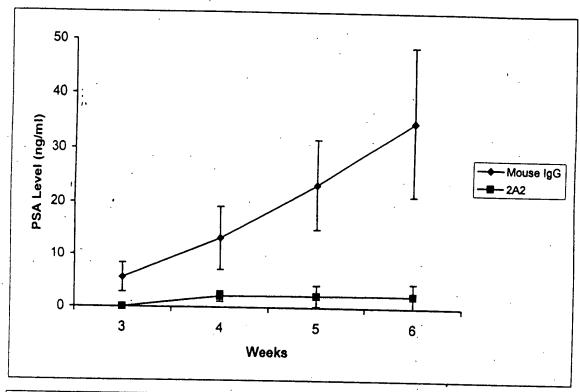


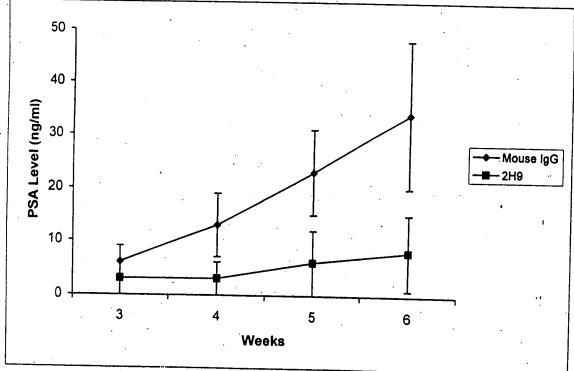




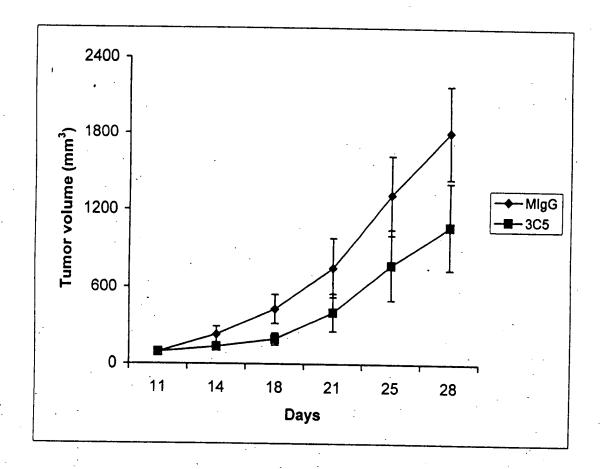












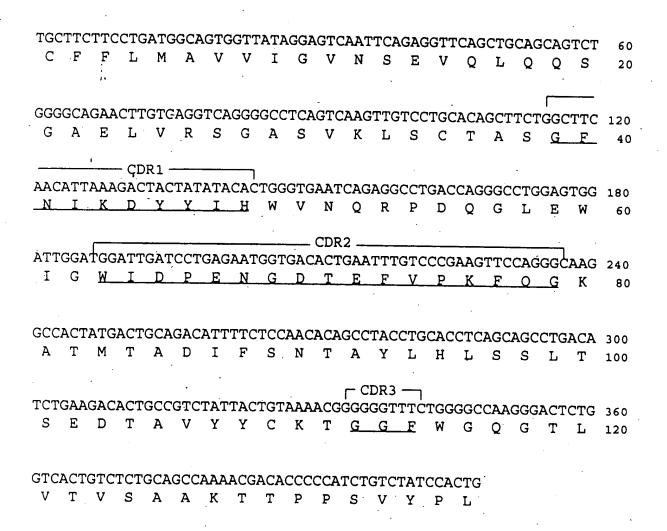


FIG. 59

TT	GGT	AGC	AAC	AGCC	TCA	GAI	GTO	CAC	CTC	CCAC	GTO	CAZ	ACTG	CAG	CAA	CCI	'GGC	TCI	GAA	60
L	V	A ;.	T	Α	S	D	٧	Н	S	Q	V	Q	L	Q	Q	P	G	S	E	20
CT	GGT	AGG	CCI	GGA	ACT	TCA	GTG	AAC	CTC	GTCC	TGC	CAAC	GCT	TCT	GGC	TAT:	'ACA	TTC	CTCC	120
L	V	R	P	G	Т	S	V	K	L	S	С	K	Α	S	<u>G</u>	<u>Y</u>	_T_	F	S R1	40
AG	CTAC	CTGG	ATO	CAC	TGG	GTG	AAG	CAC	AGG	3CCI	GGA	CAA	\GGC	CTT	'GAG	TGG	ATT	GGA	TAA	180
_ <u>s</u>	<u> Y</u>	W_	<u>M</u>	Н	W	V	K	Q	R	P	G	Q	G	Ļ	E	W	Ι	G	N_	60
AT'	TGAC	CCI	GGI	'AGI	'GGT	TAC	ACT	'AAC	TAC	CGCI	GAC	SAAC	CCTC	AAG	ACC	'AAG	GCC	'ACA	CTG	240
I_	D_	P	_G_	_S_	_G CDR		<u>T</u>	<u>N</u>	<u> </u>	_A_	<u>E</u>	_N_	L	K	<u>T</u>	K	A	T	Ĺ	80
AC	TGT <i>F</i>	GAC	CACA	TCC	TCC	AGC	ACA	.GCC	TAC	CATG	CAG	CTC	AGC	AGC	CTG	ACA	TCT	GÁG	GAC	300
Т	V	D	T	S	S	S	T	A	Y	M		L	S	S	L	Т	S	E	D	100
TC	TGC	GTC	TAT	TAC	TGT	ACA	AGC	CGA	TCI	ACT	'ATG	ATI	'ACG	ACG	GGA	TTT	GCT	TAC	TGG	360
S		V	Y	Y	C	T	S		S		M	I		T		F		Y	W	120
				,									CD	R3						
	CCAA	.GGG	ACT	CTG	GTC	ACT	GTC	TCT	GCA	GCT	'ACA	ACA	ACA	GCC	CCA	TCT	GTC	TAT	CCA	420
G	Q	G	Т	L	V	T	V	S	A	A	T	T	Т	A _.	P	S·	٧.	. Y	P	160

CTGGCC L A

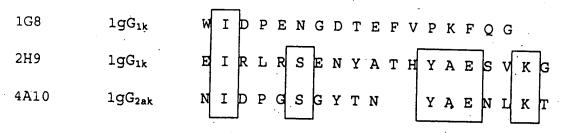
AA	TGA	CTT	CGG	GTT	GAG	CTG	GGT:	rtt.	rat'	TAT'	TGT'	TCT'	ттт	ααα	AGG	രവസ	~~~	~ > ~ /	TGAA	
N	D	F	G	L	S	W	V	F	I	I	V			K	G				E. E.	
		;.									·	_	_		G	V	K	5	E	20
CITIC	77.00	a cama		~~~	~															
GTC)AGE -	3C[]"]	'GA(3GA(GTC'	rgg <i>l</i>	AGG	AGGC	CTG	GT(GCA/	ACC'	TGG?	AGG.	ATC	CATO	GAA.	ACTO	CTCC	120
V	R	Ъ.,	E	E	S	G	G	G	W	V	Q	P	G				K	L	s	4 (
		1																		
TGT	GTA	\GCC	TCI	IGGI	ኒጥጥ	ויאכיו	ייייירי	ימכיז	רממי	מידים (ىلىرىتار.	יחיתי	ת ד	ma	3.Cm/	200			CCA	
С	V	A	S.	G	ਜ ਜ	Ť	 F	AO I	י באבה.	V IA	7.7 7.7	MI (JAC I						.'CCA	180
_			Ū	<u> </u>					R1		w_			W	V	R	Q	S	P	60
									/1(1											
GAG	AAC	GGG	CTI	GAG	TGO	GTT	GCT	'GAA	ATT	CGA	\ ጥ ፐር	AGZ	ייטעי	ז מ בוי	יא א	חי א יחים		202	CAT	
E	K	G	L	Ε	W	V	Α	E	I	R	T.	R	S	다	אנ ארטיני	A LIWI	. GCA	MCA	H	240
															CDF					80
														•						
TAT	'GCG	GAG	TCT	'GTG	AAA	\GGG	AAA	TTC	ACC	ATC	TCA	AGA	GAT	'GA'I	TCC	AGA	АСТ	ירכיי	CTC	300
<u>_Y</u>	_A_	E	S	V	K	G	K	F	Т	I	S	R	D	D	S	R	s	R	T,	
													-	_		10	5	K	ш	100
	,					•						•			•					
TAC	CTG	CAA	ATG	AAC	AAC	ATT:	AGA	CCT	GAA	GAC	AGT	GGA	ATT	TAT	TAC	TGT	ACA	СДТ	GGT	260
Y	L	, Q -	M	N	N	L	R	P	E	Ď	S	G		Y		С		D		120
										•		•					. •		<u> </u>	120
																			•	
CTG	GGA	CGA	CCT.	AAC	TGG	GGC	CAA	GGG	ACT	CTG	GTC.	ACT	GTC'	TCT	GCA	GCC	AAA.	ACG:	ACA	420
<u> </u>	<u> </u>	К_	Р_	_N	W	Ģ	Q	·G	T	L	Ÿ	T	V		A	A	K	т Т	T	140
	C	DR3										•						-	•	T-4.0
							•			٠.						•				
CCC	CCA'	rcr(3TC'	TAT(CCA	CTG	GCC	CTT	rgt(GTA									-	

that and other if that allo

CDR1 Comparisons

1G8	; .		Middle										
2H9 (1gG _{1k}	N-Term.	G	F	T	F	S	N	Y	W	M	Т
4A10	ı	1gG _{2ak}	N-Term.	G	Y	Т	F	s	S	Y	W	M.	н

CDR2 Comparisons



CDR3 Comparisons

1G8	$1gG_{1k}$	G	G	F							
2H9	$1gG_{1k}$	L	G	R	P	N					•
4A10	$1gG_{2ak}$	R	S	T	М	I	Т	T	G	F	A Y

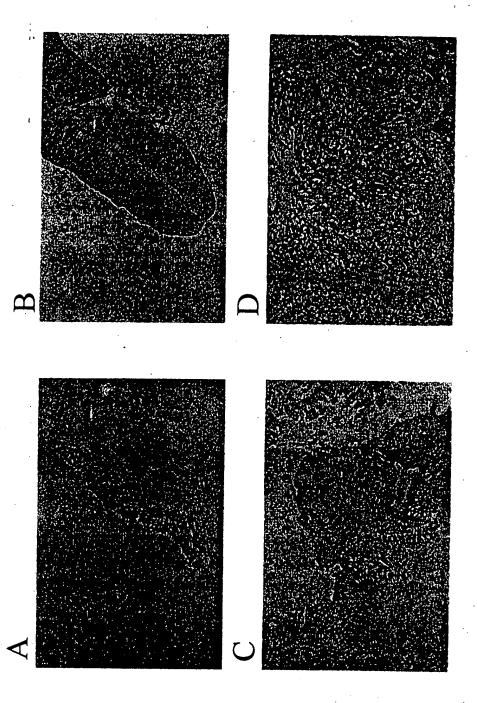
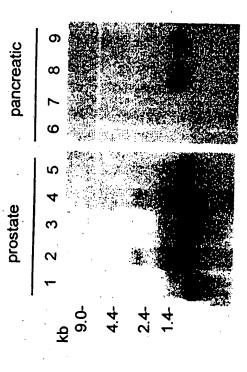
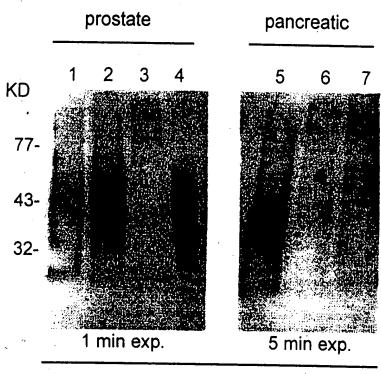


FIG. 63



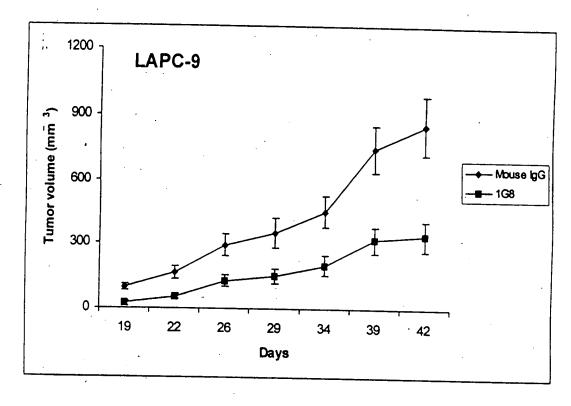
1. Prostate 6. PANC-1 2. LAPC-4 AD 7. BxPC-3 3. LAPC-4 AI 8. HPAC 4. LAPC-9 AD 9. Capan-1 5. LAPC-9 AI

FIG. 64



anti-1G8

- 1. LAPC-4 AD
- 2. LAPC-9 AI
- 3. LNCaP
- 4. LNCaP-PSCA
- 5. HPAC
- 6. Capan-1 7. ASPC-1



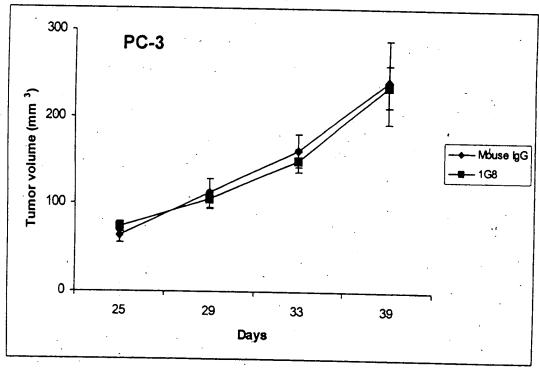
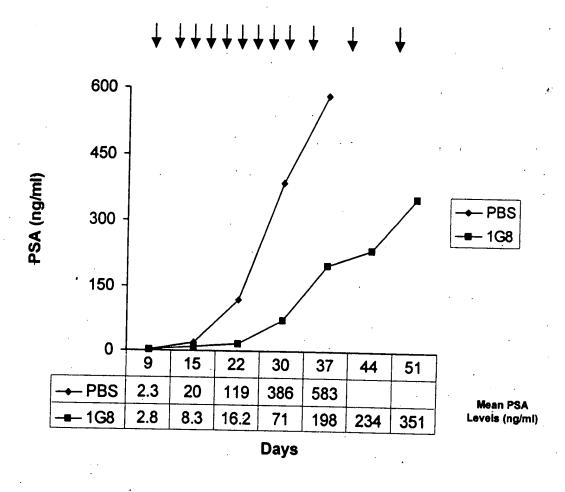


FIGURE 65

B)



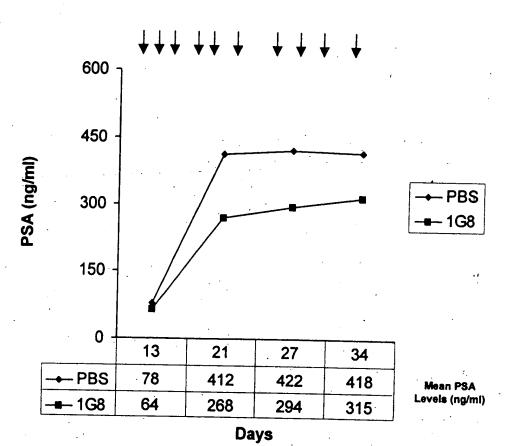
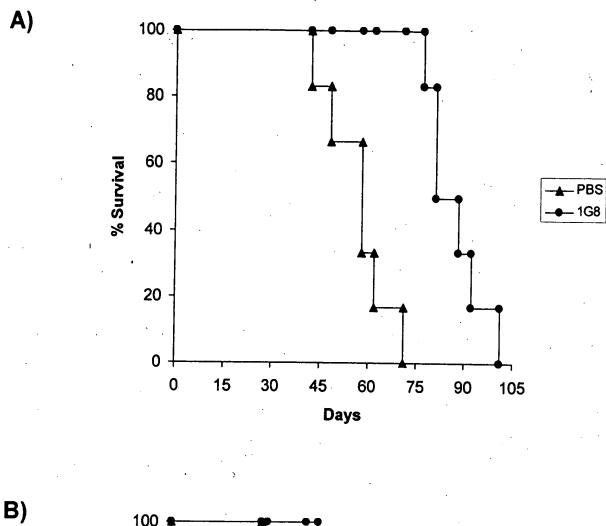
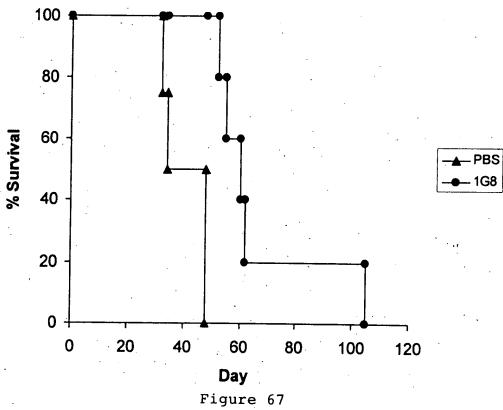
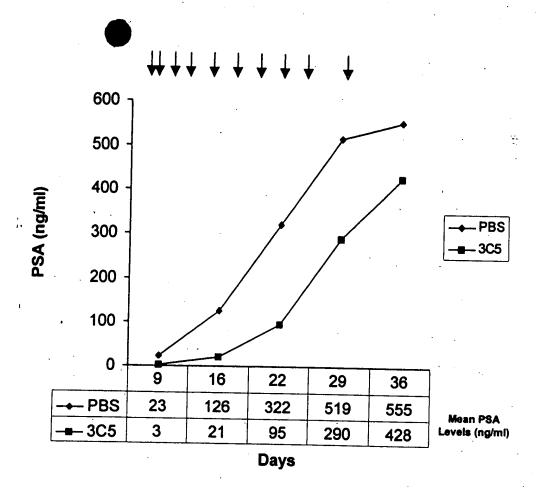


Figure 66





B)



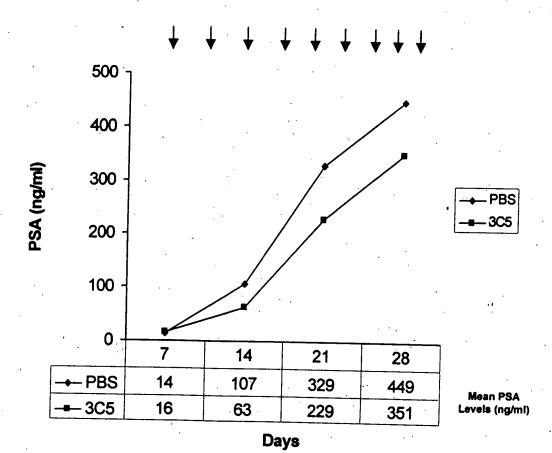
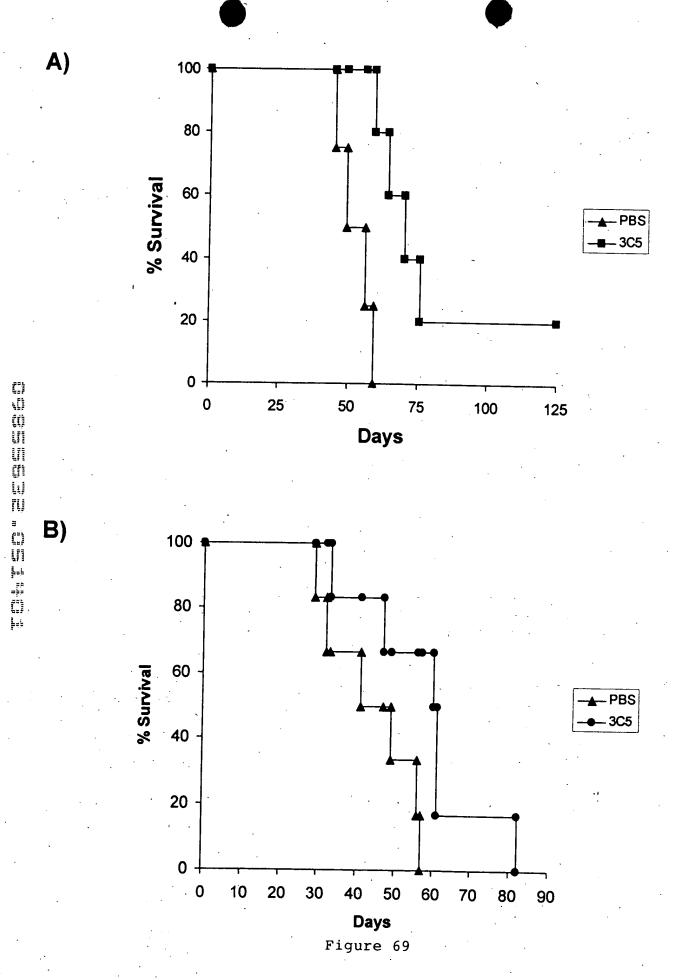


Figure 68



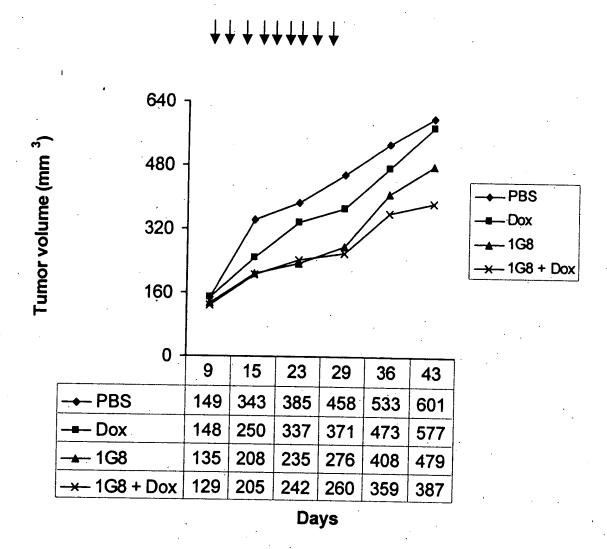


Figure 70

PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

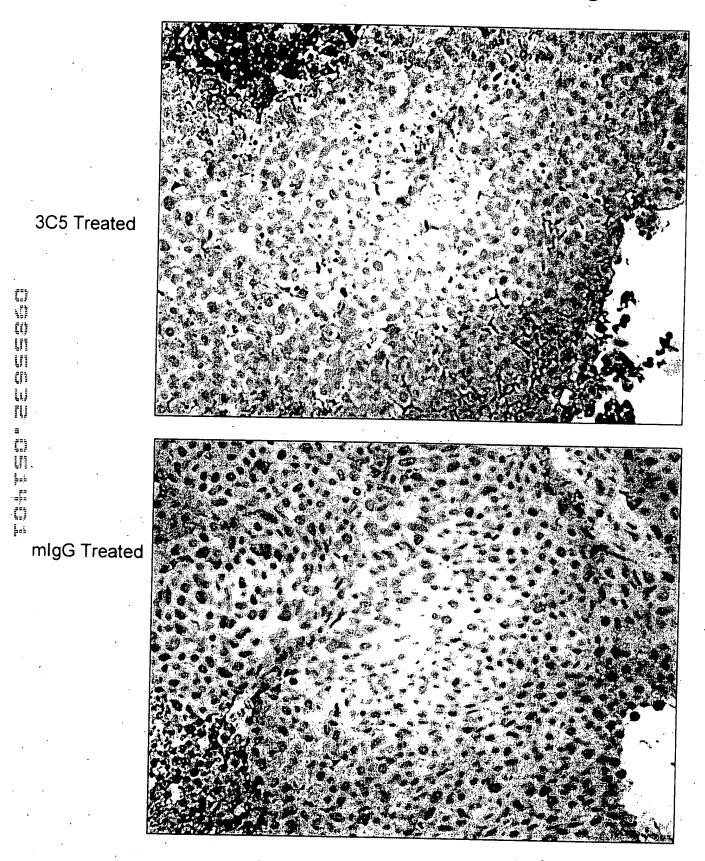
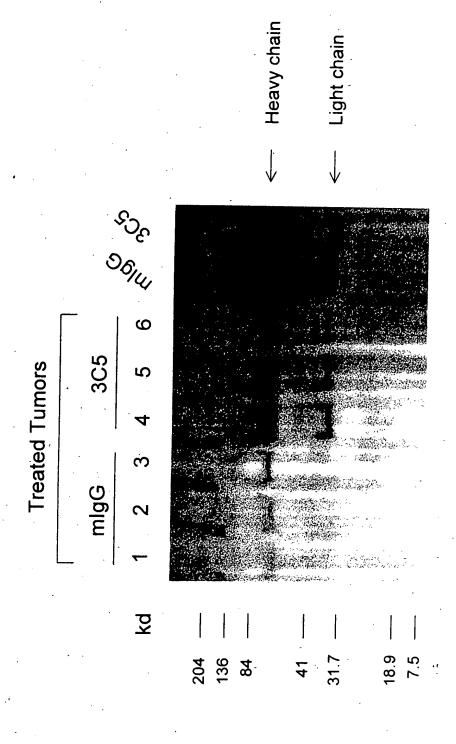


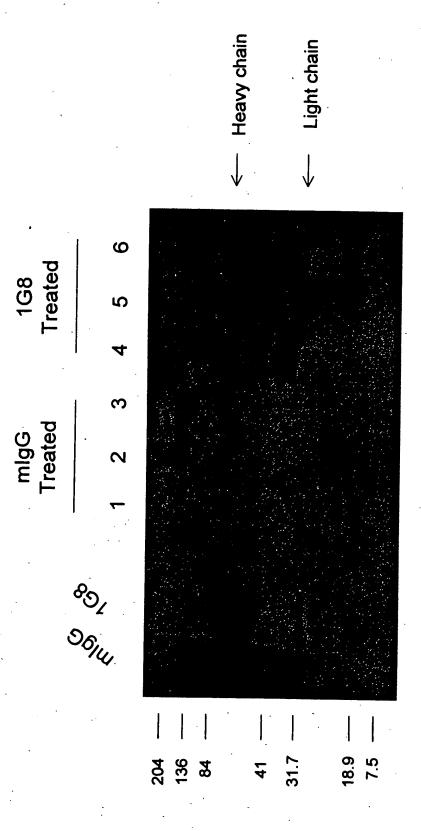
Figure 71

3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors



Western blot developed with $\alpha\text{-mlgG/k}$

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAB **TO ESTABLISHED LAPC-9 TUMORS**



- α-MigG Western

M thod: Mice bearing established LAPC-9 tumors (>100 mm³) were injected with either mlgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.